IN THE SPECIFICATION

Page 1, Please add the following paragraph before the paragraph beginning on page 1, line 1.

This application is a Division of U.S. Application No.: 09/674,866, filed June 4, 2001, which is the National Stage of International Application PCT/EP99/03244, filed May 6, 1999.

Page 1, between lines 2 and 3, please insert the heading

Field of the Invention

Page 1, between lines 6 and 7, please insert the heading

Background of the Invention

Page 2, between lines 10 and 11, insert the heading

Detailed Description of the Invention

Page 4, line 7, Please replace the paragraph beginning on page 5, line 4, with the following rewritten paragraph:

Thus, in a first aspect the invention provides for a ~~nucleotide sequence~~ nucleic acid comprising the nucleotide sequence depicted in SEQ ID NO 1 encoding the structural proteins and part of NSP4 of the PD virus, fragments of said nucleotide sequence and a ~~nucleotide sequence~~ nucleic acid comprising the nucleotide sequence depicted in SEQ ID NO 14. Preferred fragments of the nucleotide sequences according to the invention are nucleotide

fragments 1222-5076 (also referred to as p130 encoding the capsid, E3, E2, 6K and E1 proteins), 2068-5076 (also referred to as p98 encoding the E3, E2, 6K and E1 proteins), 2068-3594 (also referred to as pE2 encoding E3 and E2 proteins), 1222- 15 2067 (capsid), 2068-2280 (E3), 2281-3594 (E2), 3595-3690 (6K), and 3691-5076 (E1). For the purpose of this invention the nucleotide sequences according to the present invention also encompass the nucleotide sequence depicted in SEQ ID NO 1 and fragment sequences thereof (such as the p 130 and p98 fragments) which at least comprise a nucleotide sequence encoding for a 6K protein, wherein the nucleotide sequence depicted by nucleotide 3595-3690 of SEQ ID NO 1 has been substituted with the nucleotide sequence depicted in SEQ ID NO 14. Also within the scope of this invention are ~~nucleotide sequences~~ nucleic acids comprising tandem arrays of the nucleotide sequence comprising the sequence depicted in SEQ ID NO 1 or SEQ ID NO 14 or fragments thereof. Nucleotide sequences that are complementary to the sequence depicted in SEQ ID NO 1, SEQ ID NO 14, or parts thereof are also within the scope of the invention, as well as nucleotide ~~sequence~~ sequences that hybridise with the sequence depicted in SEQ ID NO 1 or SEQ ID NO 14. The hybridisation conditions for this purpose are stringent, preferably highly stringent. According to the present invention the term "stringent" means washing conditions of 1 x SSC, 0.1% SDS at a temperature of 65òC; highly stringent conditions refer to a reduction in SSC towards 0.3 x SSC.

Page 5, line 3, Please replace the paragraph beginning on page 5, line 3, with the following rewritten paragraph:

Nucleotide sequences that hybridise with the sequence shown in SEQ ID NO 1 or SEQ ID NO 14 are understood to be nucleotide sequences that have a sequence homology of at least 70%, preferably 80%, and more preferably 90% with the corresponding matching part of the sequence depicted in SEQ ID NO 1 or SEQ ID NO 14. According to the present invention the sequence homology is determined by comparing the nucleotide sequence with the corresponding part of the sequence depicted in SEQ ID NO 1 or SEQ ID NO 14. The sequence homology between a to nucleotide and the sequence in SEQ ID NO 1 or SEQ ID NO 14 can be determined via common sequence analysis program such as BLASTN and the like. The optimal match area is automatically determined by these programs. Homologous sequences can easily be isolated from closely related PD virus strains with the sequence depicted in SEQ ID NO 1 or SEQ ID NO 14, or fragments of these sequences, using routine cloning and hybridisation techniques. Sleeping Disease (SD) virus is closely related to PD virus and the nucleic acid sequences encoding the structural capsid, E3, E2, E 1 and 6K proteins of SD virus have the ~~necessary~~ necessary sequence homology with the nucleic acid sequences depicted in SEQ ID NO 1 and 14. Thus, these SD nucleic acid sequences are also within the present invention.

Page 5, line 20, Please replace the paragraph beginning on page

5, line 20, with the following rewritten paragraph:

The nucleotide sequences of the invention can be used in the preparation of a DNA vaccine to vaccinate fish against PD infection. DNA vaccination refers to the induction of an immune response to one or more antigens that are expressed in vivo from a gene inserted in a DNA plasmid which has been inoculated directly into the vaccinated fish. Thus, in a second aspect of the invention there is provided for a DNA vaccine comprising a ~~pharmaceutical~~ pharmaceutically acceptable carrier and a DNA plasmid in which a nucleotide sequence encoding one or more PDV structural proteins is operably linked to a transcriptional regulatory sequence.

Page 9, line 3, Please replace the paragraph beginning on page 9, line 3, with the following rewritten paragraph:

The proteins according to the invention can be prepared via standard recombinant protein expression techniques. For this purpose a nucleotide sequence encoding ~~en~~ one or more of the proteins according to the invention or a multimere of said protein is inserted into an expression vector. Preferably the nucleotide sequence is a nucleotide sequence comprising the nucleotide sequence depicted in SEQ ID NO 1 or SEQ ID NO 14 or one or more fragments of these sequences. Preferred fragments of the nucleotide sequences according to the invention are nucleotide fragments 1222- 5076, 2068-5076, 2068-3594, 1222-2067, 2068-2280, 2281-3594, 3595-3690 3691- 5076 of the sequence

depicted in SEQ ID NO 1, and combinations thereof such as, for example, fragment 1222-2067 with fragment 2281-3594. Further preferred fragments according to the invention are fragments of the nucleotide sequence depicted in SEQ ID NO 15 such as, for example, the nucleotide sequence depicted by nucleotides 3595-3690 of SEQ ID NO 1. Also suitable are nucleotide sequences that are complementary to the sequence of SEQ ID NO 1 or SEQ ID NO 14 or nucleotide sequences of which the sequence homology with the sequence depicted in SEQ ID NO 1 or SEQ ID NO 14 is at least 70%, preferably 80%, and more preferably 90%. The sequence homology between the nucleotide sequences that are suitable for use in the DNA plasmid is determined as described earlier.

Page 10, line 1, Please replace the paragraph beginning on page 10, line 1, with the following rewritten paragraph:

The invention furthermore provides for a vaccine comprising one or more of the structural PD proteins and a ~~pharmaceutical~~ pharmaceutically acceptable carrier. More specifically, a vaccine according to the invention comprises a capsid protein having an amino acid sequence depicted in SEQ ID NO 4 or a derivative thereof, an E3 protein having an amino acid sequence depicted in SEQ ID NO 5 or a derivative thereof, an E2 protein having an amino acid sequence depicted in SEQ ID NO 6 or a derivative thereof, an E1 protein having an amino acid sequence depicted in SEQ ID NO 8 or a derivative thereof, a 6K protein having an amino acid sequence depicted in SEQ ID NO 7 or SEQ ID NO 15 or a

derivative thereof, or a mixture comprising two or more of the to proteins according to the invention. Preferably the vaccine according to the invention comprises the E2 protein, and optionally the capsid protein. Also preferred is a vaccine comprising all structural proteins of PD; these proteins can spontaneously form virus-like particles, thus providing a vaccine that closely resembles that of the whole pathogen. Vaccines according to the invention are suitable for use as a marker vaccine to distinguish between vaccination and infection by PD in the field. A preferred vaccine according to the invention is a marker vaccine comprising a 6K protein having the amino acid sequence depicted in SEQ ID NO 7.

Page 10, line 23, Please replace the paragraph beginning on page 10, line 23, with the following rewritten paragraph:

Vaccines according to the invention comprise an effective amount of the afore-mentioned DNA plasmids, vector bacteria or virus, or proteins and a ~~pharmaceutical~~ pharmaceutically acceptable carrier. The term "effective" as used herein is defined as the amount sufficient to induce an immune response in the target fish. The amount of plasmid, vector or protein will depend on the type of plasmid or vector, the route of administration, the time of administration, the species of the fish as well as age, general health and diet.

Page 11, line 4, Please replace the paragraph beginning on page 11, line 4, with the following rewritten paragraph:

~~Pharmaceutical~~ Pharmaceutically acceptable carriers that are suitable for use in a vaccine according to the invention are sterile water, saline, aqueous buffers such as PBS and the like. In addition, a vaccine according to the invention may comprise other additives such as adjuvants, stabilisers, anti-oxidants and others.

Page 12, Please replace the subject heading beginning on page 12, line 11, with the following rewritten subject heading:

~~LEGEND~~ Brief Description of the Figures

Page 17, line 17, Please replace the paragraph beginning on page 17, line 17, with the following rewritten paragraph:

A ~~standardised~~ standardized challenge experiment performed at 8 weeks post- vaccination in Atlantic salmon fish showed that protection against challenge with salmon PD virus could be obtained with these recombinant sub-unit vaccines. In the experiment, 2 0 lesions in pancreas, skeletal muscle and heart muscle were scored in ~~ordinal~~ ordinary way. Significant levels were calculated from Kruskal-Wallis one-way analysis of variance (non-parametric test). The vaccine formulation comprising the E2 or E2- E3 proteins gave similar levels of protection as obtained

by the inactivated PD virus vaccine, while vaccines containing the recombinant proteins resulting from the p130 and p98 constructs respectively were less protective then the ~~inactivate~~ inactivated PD virus vaccine.

Page 17, line 27, and ending page 18, line3, Please replace the paragraph beginning on page 17, line 27, with the following rewritten paragraph:

DNA vaccination with proteins obtained from expression of the p130 nucleotide construct was carried out in mice to test for the antigenic properties of the recombinant proteins. After two ~~imntramuscular~~ intramuscular inoculations with p130- pcDNA3.1 recombinant expression plasmids (see clone 1), the sera of mice showed an antibody reaction with in vitro produced PD virus.